Research Article

Investigation of Spectrophotometric for Determination of Cloxacillin Sodium in Different Brands of Pharmaceutical Preparations

Sajjad Ali Ilyas[a], Muhammad Imran[b,c,*], Naresh Kumar[c,d], Jasmin Shah[a*], M. Rasul Jan[a]
[a]Institute of Chemical Sciences, University of Peshawar, Peshawar, Pakistan.
[b]Department of Chemistry, University of Azad Jammu & Kashmir, Muzaffarabad, Pakistan.
[c]UCG Thar Project, Islamkot, Sindh, Pakistan.
[d]Institute of Chemistry, University of São Paulo, São Paulo, Brazil.
***Corresponding author's E-mail:** imjaral@yahoo.com

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ABSTRACT

This article comprises a very effective, sensitive economical and precise spectrophotometric method for the fortitude of cloxacillin sodium in different brands of pharmaceutical preparations. Studied method is based on complexation of cloxacillin sodium with Cu ions by heating in water bath. The method has investigated the parameters of wavelength 450nm concentration of Cu ions (1000 ppm) and volume of the Cu ions 20 mL with incubation time of 10 minutes, for 40 ppm of cloxacillin sodium. The limit of detection was found 0.3099 with quantification limit of 1.033. The standard deviation was found 0.1033, and relative standard deviation of 1.033. The method was applied and found very promising for the determination of cloxacillin sodium in various pharma brands with labeled claims of the assay. The investigated method is recommended for the quality control analysis of the cloxacillin sodium in pharma industry.

Keywords: Spectrophotometric method, Cloxacillin sodium, Pharmaceutical Preparations.

INTRODUCTION

Cloxacillin is semi-synthetic antibiotic related to penicillin,[1] and is also isoxazolyl penicillin.[2] Cloxacillin is used in treatment of infections due to staphylococci resistance to benzyl penicillin. It is administered by orally or by injection as sodium salt.[2]

Cloxacillin is a bactericidal with mode of action similar to benzyl penicillin. It's active against pancillinase- producing and non-pencillinase-producing staphylococci. It's activity against streptococci such as Streptococcus pneumonia and str. Pyogenes is less than that of benzylpenicilline, but sufficient to be useful when these organisms are present with penicillin resistant staphylococci. Cloxacillin is virtually ineffective against Enterococcus faecalis Resistance.[2]

Cloxacillin is incompletely absorbed from the gastrointestinal tract, after an oral dose of 500mg, peak plasma concentration of 7-15 µg/mL in fasting subjects in 1-2 hours. Absorption is more complete when given intramuscular injection and peak plasma concentration of about 15 µg/mL is observed in 30 minutes after a dose of 500mg. drug has been reported to have plasma half life of 0.5-1 Hour.[2]

The chemical structure of Cloxacilin Sodium is shown in Figure 1.[3-5]

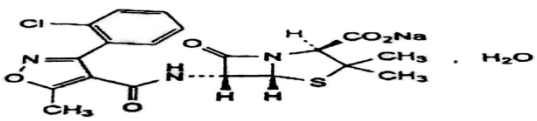

Figure 1: The structural formula of cloxacillin sodium

Chemical formula: $C_{19}H_{17}ClN_3NaO_5S$, H_2O.[4,5]

Molecular weight: 475.9[4,5]

Characters

A white, hygroscopic, crystalline powder, soluble in methanol, alcohol and freely soluble in water. In the present study an attempt was made to develop an innovative, less expensive, a simple and more authentic method for the determination of Cloxacilin Sodium in pure state and its pharmaceutical preparations.[5]

Spectrophotometric determination method development strategy for Cloxacilin sodium

The presence of carboxylic acid functional group in the formula of drug suggests the possibility of complexation with transition metal offering a possibility for its spectrophotometric determination. Therefore in the proposed method Cloxacilin sodium was tried for its complex formation with various transition metals such as Cr, Ni, V, Cu to give a colored product. The colored product thus formed was used for the determination of Cloxacilin sodium.

MATERIALS AND METHODS

Preliminary Study of the chelation potential for spectrophotometric determination of Cloxacilin sodium

Preliminary determinations were conducted to explore the possibility of the formation of expected colored complex between metal ion and Cloxacilin sodium. Initially higher concentration of Cloxacilin sodium (1000ppm) was treated with different metal ion solution along with heating to check the formation of expected

colored product. The colored product indicates the chances of reaction and subsequent formation of Cloxacilin sodium complex by this method. Further studies were focused on the optimization of various parameters for maximum complexation and are discussed below.

Investigation of appropriate wavelength for the estimation of Cloxacilin metal complex

Instruments

Digital analytical balance, thermostatic water bath and UV/VIS spectrophotometer were used during this investigation.

Reagents

Analytical reagent grade copper nitrate trihydrate and Cloxacilin sodium were used during this work.

Solution Preparation

Metal Ion Solution

Metal Ion solution (1000ppm) was prepared by dissolving 0.380g of $Cu(NO_3)_2.3H_2O$ in distilled water and diluted to 100 mL with distilled water.

Standard Cloxacilin Sodium solution

Cloxacilin sodium (1000ppm) stock solution was prepared by dissolving 0.1 g of authentic standard Cloxacilin sodium in distilled water and diluted upto 100 mL with distilled water.

Procedure

Cloxacilin sodium 2 mL from (1000 pm) stock solution was slowly transferred to 50 mL volumetric flask. To this 10 mL of metal ion solution from (1000 ppm) stock solution of metal ion was added with little dilution with water followed by incubation in boiling water bath for 20 minute. The contents were allowed to cool to room temperature in tape water tub, and diluted to 50 mL with distilled water. Blank solution was prepared by the same procedure without addition of Cloxacilin sodium. The absorbance of yellow colored complex was measured from 400-570 nm, using Genesys 5 spectrophotometer. Each time after changing wavelength the instrument calibrated with blank solution.

The results are given in Table 1 and are shown in Figure 2.

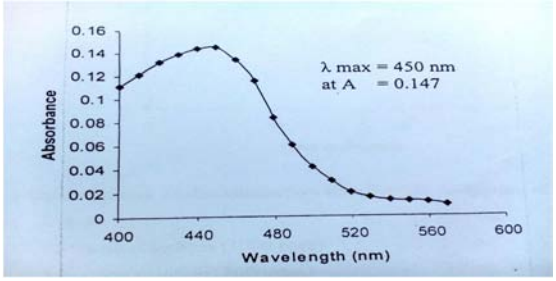

Figure 2: Wavelength optimization for spectrophotometric determination of cloxacillin sodium

Investigation of the effect of metal ion solution and incubation time on the formation and absorbance behavior of complex.

Instrument: The same as mention before

Reagent: The same as mention before

Solution: The same as mention before

Procedure

Standard Cloxacilin sodium solutions 2 mL from (1000 ppm) stock solutions were transferred to six separate 50 mL volumetric flasks and to each of these flasks, varied volumes of metal ion solution from (1000 ppm) stock solution was added along with little dilution with distilled water and was incubated in water bath for 20 minute. The resulting colored complex was allowed to cool and diluted to 50 mL with distilled water. Blank was prepared in the same manner without the addition of Cloxacilin sodium. The absorbance of the resulting complex was measured at 450 nm using Genesys 5 spectrophotometer. The results are given in Table 2 and are shown in Figure 3. For studying the effect of incubation time Standard Cloxacilin sodium solution 2 mL from 1000 ppm stock solution was transferred to six separate 50 mL volumetric flasks. To each of these flask 20 mL of metal ion solution form stock solution and little volume of water was added followed by incubation in boiling water bath for varied times, in a range of 0-25 minutes. The resulting yellow colored complex was cooled and diluted to 50 mL with distilled water. Blank was prepared in the same manner without the addition of Cloxacilin sodium. The absorbance of the resulting complex was measured at 450 nm using Genesys 5 spectrophotometer. The results are given in Table 3 and are shown in Figure-4.

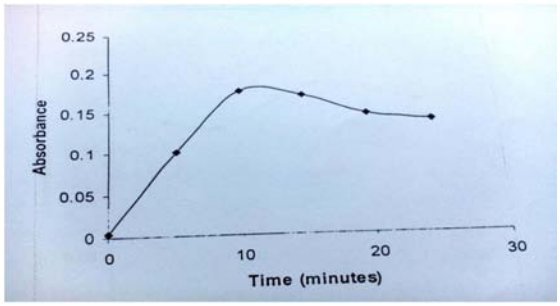

Figure 3: Optimization of the incubation time for the formation of complex

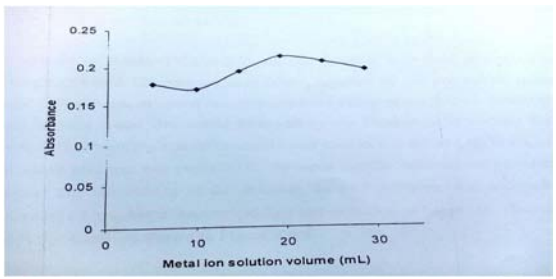

Figure 4: Optimization of volume of metal ion solution for formation of complex

The effect of concentration on the absorbance behavior of Cloxacillin Cu complex

Instruments: The same as mentioned before

Reagents: The same as mentioned before

Solutions: The same as mentioned before

Procedure

Varied amount of standard Cloxacilin sodium solution with final concentration after dilution ranging from 2-120 ppm taken in fifteen separate 50 mL volumetric flasks. To each of these flask 20mL of metal ion solution from (1000 ppm) stock solution and little volume of distilled water was added followed by the incubation in boiling water bath for 10 minutes. The resulting colored complex was cooled and diluted to 50 mL with distilled water. Blank solution was prepared in same manner without the addition of Cloxacilin sodium. The absorbance of the resulting colored complex was measured at 450 nm using Genesys 5 spectrophotometer to find out absorbance behavior. The results are given in Table 4 and are shown in Figure 5.

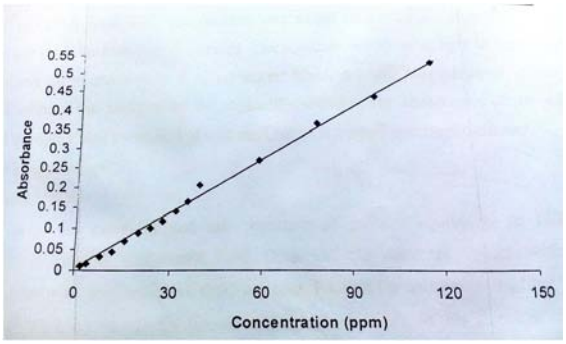

Figure 5: The effect of concentration on the absorbance behavior for cloxacillin sodium at lower concentration using spectrophotometric method

Analysis of Cloxacilin sodium in various pharmaceutical preparations using investigated method and its comparison with official method

Instruments: The same as mentioned before

Reagents: The same as mentioned before

Solutions: The same as mentioned before

Procedure

Accurately weigh 100 mg of reference standard cloxacillin sodium in 100 mL volumetric flask. Dissolved it in distilled water by continues shaking and made up to mark. Pipette out 3 mL of the solution into 50 mL volumetric flask, to this 20 mL of (1000 ppm) Cu^{++} ion solution was added after little dilution and incubation was carried out in water bath for 10 minutes. The solution was allowed cooling in tape water tub and diluted upto mark with distilled water. Blank solution was prepared in the same manner without the addition of Cloxacilin sodium. The absorbance of the resulting colored complex was measured at 450 nm using Genesys 5 spectrophotometer.

Sample preparation

Procedure

Weigh 20 capsules and take contents of powder equivalent to 100 mg cloxacillin in 100 mL volumetric flask. Dissolved and made the volume with distilled water, shaked well sonicated for 5 minutes. Filtered the solution using filter paper # 1, Pipette out 3 mL of the filtrate into 50 mL volumetric flask, to this 20 mL of (1000 ppm) Cu^{++} ion solution was added along with little volume of water followed by incubation in water bath for 10 minutes, allow it to cool and diluted upto mark by distilled water and measure the absorbance at 450 nm. In cause of injection proceed same as for standard preparation.

Calculations

$$\text{Mg/caps/injection of cloxacillin} = \frac{\text{Au x wt of std x 3 x100 x 50 x 5 x potency of standard x average weight}}{\text{As x 100 x 50 x Wt of sample x 3 x 100}}$$

Where Au= Absorbance of Sample

As= Absorbance of Standard

Percentage Label Claim = (mg per caps/inj x 100) / (L.C mg per caps/inj)

Determination of cloxacillin sodium by official method (HPLC method)

Buffer

Prepared a 0.2 M solution of monobasic potassium phosphate in water, and adjusted with 2N NaOH to pH of 6.8.

Mobile phase

Prepared a mixture of buffer and acetonitrile (80:20).

Standard preparation

Prepared a solution of USP cloxacillin sodium in buffer having a concentration of 0.55 µg/mL.

Sample Preparation

Transferred 110 mg of cloxacillin sodium in 200 mL volumetric flask diluted with buffer to volume, and mixed using magnetic stirrer for five minutes to dissolve.

Chromatograph parameters

Wavelength	:	225 nm
Flow rate	:	1 mL/min
Column	:	4.6 mm x 25 cm (Contains packing L1)

Procedure

Separately injected equal volume (20 microlitres) of standard and sample into the 200(CE / W) (ru / rs)

C = concentration in mg/ mL

E = cloxacillin equivalent, in microgram per mL

W = weight in mg

ru and rs = cloxacillin peak response obtained from assay and standard preparation respectively.

RESULTS AND DISCUSSION

The proposed method involves the complexation of the cloxacillin sodium with Cu^{++} ions leading to a yellow colored complex.

Various parameters like wavelength, volume of Cu^{++} ion solution, incubation time were optimized for the formation of utmost colored complex.

After the preliminary experiment the complex formed, was investigated for the optimal wavelength. The results are given in Table 1 and are shown in Figure 2 as can be seen from Table 1 that resulting complex has a maximum absorbance at 450 nm, and was used as optimum wavelength for further investigation of Cloxacillin sodium determination.

Cu^{++} solution (1000 ppm) was used for the complexation. Various volumes of (1000 ppm) Cu^{++} ion solution in the range of 5 to 30 mL were tried with 40 ppm of cloxacillin sodium. The results are given in Table 2 and are shown in Figure 3. As can be seen from the Table 2 that 20 mL of Cu^{++} ion (1000 ppm) solution was found to be optimum volume for the formation of maximum complex with 40 ppm of cloxacillin sodium.

It was observed that incomplete complexation could be achieved at room temperature and it was necessary to heat the solutions in hot water bath. Therefore incubation time in range of 0-25 minute was investigated for maximum complexation in boiling water bath and the results are given in Table 3 and are shown in Figure 4. It was found that 10 minute incubation time in boiling water bath was the optimum incubation time for maximum complexation.

Effect of concentration at lower level on the absorbance behavior of cloxacillin Cu^{++} was investigated to calculate the limit of detection (LOD) and limit of quantification (LOQ) at optimum conditions. Cloxacillin sodium 2 ppm was selected for investigation of detection limit as this was the minimum concentration for which the absorbance could be noted. Six replicate readings were taken for this concentration. The results are given in Table 6. The following formulas were used for calculation of LOD, LOQ, S.D and R.S.D.

Limits of detection (for concentration) = 3 x S

Limits of quantifications (for concentration) = 10 x S

Standard deviation. $S\sqrt{n/(n-1)}$

Relative standard deviation. R.S.D= S/X^{-} x 100

Where as

X = Concentration in (ppm) found.

X^{-} = Average founded concentration (ppm) of six samples.

n= X- X^{-}

Applications of investigated method for the determination of cloxacillin sodium in different pharmaceutical preparations and comparison with official method

The investigated method was applied for determination of cloxacillin sodium in different formulations of capsules and injections. The method used for determination and calculations has been discussed above and was compared with official method, while the results of comparison have been shown in Table 7.

Table 1: Wavelength optimization for spectrophotometric determination of cloxacillin sodium

Wavelength	Absorbance	Wavelength	Absorbance
400	0.111	490	0.061
410	0.122	500	0.042
420	0.133	510	0.030
430	0.140	520	0.020
440	0.146	530	0.016
450	0.147	540	0.014
460	0.136	550	0.013
470	0.118	560	0.012
480	0.085	570	0.010

Table 2: Optimization of the incubation time for the formation of complex

Time in	0.00	5.0	10.00	15.00	20.00	25.00
Absorbance	0.004	0.101	0.179	0.172	0.148	0.139

Table 3: Optimization of volume of metal ion solution for formation of complex

Volume used in mL (1000 ppm)	05	10	15	20	25	30
Absorbance	0.178	0.172	0.197	0.218	0.211	0.200

Table 4: The effect of concentration on the absorbance behavior for cloxacillin sodium at lower concentration using spectrophotometric method

Cloxacillin sodium Conc. (ppm)	2	4	8	12	16	20	24	28
Absorbance	0.012	0.017	0.034	0.045	0.070	0.089	0.098	0.115
Cloxacillin sodium Conc. (ppm)	32	36	40	60	80	100	120	
Absorbance	0.140	0.164	0.206	0.270	0.365	0.438	0.532	

Table 5: Replicate readings for 2 ppm concentration of cloxacillin sodium

Absorbance	Concentration (ppm) found (X)
0.012	2.2
0.011	2.0
0.012	2.2
0.012	2.2
0.011	2.0
0.012	2.2

Table 6: Results of investigated method

Linear range	1-120μg
λ max	450nm
standard deviation	0.1033
R.S.D	4.849
Correlation coefficient	0.996
Molar absorptivity	$5.04*10^{-5}$
∑	$1.08*10^{3}$
L.O.D	0.3099
L.O.Q	1.033

Table 7: Application of investigated method for the analysis of cloxacillin sodium in various pharmaceutical preparations and comparison with official method

Name of Drug	Label Claim	Develop method	Official method
Auropen capsules	250 mg/capsule	251.31±1.13 mg/ capsule	251.83±1.41 mg/ capsule
Auropen inj	250 mg/vial	2.49.8±0.59 mg/vial	250.4±0.87 mg/vial
Cloxazan Capsules	250 mg/capsule	253.2±1.64 mg/ capsule	252.9±0.92 mg/ capsule

CONCLUSION

Spectrophotometric method for the determination of cloxacillin sodium involves complexation of cloxacillin sodium with Cu^{++} ions followed by heating in water bath. Various analytical parameters like wavelength, concentration and volume of metal ion solution and incubation time in water bath were optimized for spectrophotometric determination of cloxacillin sodium and were found to be 459nm, 20 ml (1000 ppm), and 10 minutes respectively for 40 ppm of cloxacillin sodium. The limit of quantification and limit of detection for the investigated method were calculated using authentic reference standard and were found to be 1.033 and 0.3099 respectively. The relative standard deviation and standard deviation were established to be 1.033 and 0.1033 respectively.

The method was found linear in range of 1-120 ppm. The developed method was effectively useful for determination of cloxacillin sodium in various pharmaceutical formulations and method was found in good conformity with labeled claim in pharmaceutical preparations.

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REFERENCES

1. "The Merck Index. An encyclopedia of chemicals, Drugs and biological" 15th ed. White house station: Merck and Co, Inc, NJ, U.S.A. 432, 2013.
2. S. C. Sweetman. "Martindail the complete drug reference" .34th ed. London, Chicago (P_hP). 198, 213, 2005.
3. USP 37 NF32. "The united state pharmacopeia The national formulary" volume 3. The united state pharmacopeial convention 12609 twin brook parkway, Rockville, MD 20852. 2438, 2014.
4. "British pharmacopoeia" Volume I. The stationery office London. I-594- I-595, 2014.
5. European pharmacopoeia" 7th ed. Volume 2. Council of Europe 67075 Strasbourg Cedex, France. 1735-1736, 2010.

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